原 著

Interactions between Inhalation Anesthetics and β-Adrenergic Receptor Signaling Pathways on L-Type Ca^{2+} Currents in Rabbit Ventricular Cardiomyocytes

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Abstract

We studied the interaction of a β-adrenergic receptor agonist and an adenylyl cyclase activator with volatile anesthetics in combination with nitrous oxide (N_2O). The whole-cell patch clamp technique was used to measure the amplitude of L-type Ca^{2+} channel current ($I_{Ca,L}$) in rabbit ventricular myocytes. The effects of sevoflurane or halothane in combination with N_2O were compared with their effects in the presence of β-adrenoceptor stimulation by isoproterenol or adenylyl cyclase activation by forskolin. The effects of sevoflurane plus N_2O and halothane plus N_2O under basal conditions significantly differed from their effects in the presence of isoproterenol. Isoproterenol and forskolin diminished the ability of two gas combinations to suppress $I_{Ca,L}$. Our findings suggest that both sevoflurane and halothane in the presence of N_2O modulate the catecholamine sensitivity of the β-adrenergic cascade.

Key words; L-type Ca^{2+} currents, ventricular myocytes, β-adrenergic receptor agonist, inhalation anesthetics

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Introduction

The L-type Ca^{2+} channel current ($I_{Ca,L}$) in mammalian ventricular myocytes controls changes in cellular calcium during excitation-contraction coupling[1]. β-Adrenergic receptor agonists with positive inotropic actions activate adenylate cyclase through binding to the G protein to increase cyclic AMP and subsequently increase $I_{Ca,L}$ through protein kinase A. On the other hand, inhibition of $I_{Ca,L}$ by inhalation anesthetics, which exhibit negative inotropic actions, has been demonstrated using patch clamp in ventricular myocytes. Although cardiac depression is a common side effect of inhalation anesthetics[2], some exert a stimulatory effect on the cardiovascular system. For example, halothane (HALO) and, to a lesser extent, other volatile anesthetics sensitize the myocardium to the arrhythmogenic effects of epinephrine[3,4]. In addition, rapid increases in the concentration of inspired desflurane can induce transient increases in heart rate and arterial pressure, reflecting sympathetic activation[5,6]. Similar increases in heart rate are observed when the concentration of inspired isoflurane is increased rapidly[6].

We previously reported the interaction of a β-adrenergic receptor agonist with isoflurane in combination with N_2O[7]. Similar to isoflurane, the depressive effect of sevoflurane (SEVO) on cardiac contrac-

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tion was less than that of HALO. We hypothesized that SEVO or HALO in combination with N_2O would interact with a β-adrenergic receptor agonist. To test this hypothesis, we examined the interaction of a β-adrenergic receptor agonist with SEVO and HALO in combination with N_2O, using the whole-cell patch clamp technique with rabbit ventricular cardiomyocytes to measure the effects on $I_{Ca,L}$.

Methods

All protocols adhered to the Guiding Principles of the Physiological Society of Japan and were approved by the Institutional Animal Experimentation Committee of Juntendo University School of Medicine.

Myocyte Isolation

Ventricular myocytes were prepared as previously described[8], with slight modifications. Japanese white rabbits (1.6–2.0 kg) were anesthetized by intravenous injection of pentobarbital sodium (75 mg/kg), administered together with heparin (1000 units) via a superficial ear vein. An additional dose of 10 mg/kg was administered if any responses to nociceptive stimuli persisted. Once the animal was anesthetized, the heart was quickly excised, washed with ice-cold Ca^{2+}-free Tyrode's solution, perfused with normal Tyrode's solution for 5 min, and then with Ca^{2+}-free Tyrode's solution for 5 min using the Langendorff method. This was followed by perfusion with collagenase solution for 30–35 min, until the atrial wall became transparent, and then by perfusion with Kraftbrühe (KB) solution for 5 min. All perfusates were oxygenated and pre-warmed to 37℃. The digested heart was minced with scissors, shaken gently for several minutes and filtered through a coarse metal filter. Filtered myocytes were stored in normal Tyrode's solution at room temperature (23–25℃).

Electrophysiological Recordings

Isolated myocytes were dispersed in a chamber mounted on the stage of an inverted microscope and superfused with normal Tyrode's solution. Whole-cell patch clamping was performed in normal Tyrode's solution at room temperature (23–25℃). Once a giga-seal was achieved, the whole-cell configuration was established by applying negative pressure to rupture the patch membrane. The myocytes were then allowed to equilibrate with the pipette solution for about 10 min. The holding potential (HP) was −80 mV. To record $I_{Ca,L}$, the normal Tyrode's solution was substituted with K^+-free Cs^+ solution. To inactivate the Na^+ current, 30 ms pre-pulses with amplitude from the HP to −40 mV were applied. To characterize the current-voltage (I-V) relationship, 300-ms depolarization steps were applied after the pre-pulses in 10 mV increments from −30 to 30 mV. To assess the time-dependent changes in peak $I_{Ca,L}$, a constant voltage depolarization pulse was applied repetitively at 0.1 Hz. Membrane currents were recorded using an EPC 7 patch clamp amplifier (List, Darmstadt, Germany). Whole-cell currents were filtered at 1 kHz using a four-pole Bessel low-pass filter and digitized at 5 kHz. Whole-cell membrane capacitance was determined either from the peak amplitude and the time constant of the current decay or from the current integral of the capacitive transients elicited by the 10-mV step pulses.

Solutions

Normal Tyrode's solution contained (mM): NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, and glucose 10 at pH 7.4 (adjusted with NaOH). $CaCl_2$ was omitted from the nominally Ca^{2+}-free Tyrode's solution. The collagenase solution contained 45 mg collagenase (Sigma, Type 1) in 50 ml of low (20μM) Ca^{2+}-containing Tyrode's solution. The KB solution contained (mM): KOH 110, taurine 10, oxalic acid 10, glutamic acid 70, KCl 25, KH_2PO_4 10, EGTA-Tris 5, Hepes-Tris 5, and glucose 10 at pH 7.4 (adjusted with KOH). The pipette solution contained (mM): CsCl 120, TEA-Cl 20, $CaCl_2$ 1, EGTA-CsOH 11, Hepes 10, and Mg-ATP 5 at pH 7.4 (adjusted with CsOH). The K^+-free Cs^+ solution in which the $I_{Ca,L}$ was recorded contained (mM): NaCl 135, CsCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, and glucose 10 at pH 7.4 (adjusted with NaOH).

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Study Protocol

After obtaining baseline recordings, during which the cells were superfused with solution aerated with air, they were superfused with solution aerated with the experimental gas mixture, and changes in $I_{Ca,L}$ were measured after allowing 90 s for the superfusate to equilibrate. The experimental gas mixtures contained 1) SEVO 0.66%, N_2O 63.5%, and O_2 36% (SEVO-N_2O); 2) HALO 0.29%, N_2O 70% and O_2 30% (HALO-N_2O). Anesthetic concentrations in the gas phase were measured using a calibrated infrared gas analyzer (Capnomac Ultima; Datex-Engstrom, Helsinki, Finland). These gases have been shown to exhibit the same anesthetic potency in humans[9,10], because the anesthetic effect of N_2O in combination with volatile anesthetic has not been confirmed in other species. To equilibrate the VA concentrations, solutions were aerated with the experimental gas mixture for more than 20 min prior to the application of command pulses. The SEVO and HALO concentrations in the superfusate were confirmed by gas chromatography (GC-17; Shimadzu Corporation, Kyoto, Japan) using a headspace sampler (Turbo Matrix HS-40; PerkinElmer, Ltd., MA, USA). The SEVO concentrations were 0.15 mM in the SEVO-N_2O solutions, while the HALO concentrations were 0.21 mM in the HALO-N_2O solutions. These concentrations are consistent with those used previously[11,12]. Isoproterenol (1 mM, ISO) and forskolin (10 mM, FSK) solutions were aerated with experimental gases before application to the chamber. The $I_{Ca,L}$ were expressed as percentages of the peak $I_{Ca,L}$ recorded under control conditions before the application of any drugs. In the presence of ISO or FSK, the $I_{Ca,L}$ were expressed as percentages of the peak $I_{Ca,L}$ obtained before application of the experimental solutions.

Data analysis and Statistics

Data acquisition and analysis were conducted using Chart Ver. 5 (ADInstruments, NSW, Australia) and IGOR Pro Ver. 4 (Wave Metrics, Oregon, U.S.A.). Peak $I_{Ca,L}$ amplitude was estimated as the difference between the maximal inward current and zero current. Current densities (pA/pF) were calculated by dividing the current amplitudes by the membrane capacitance. To allow comparisons, the data were expressed as percentages of the control data and as the means ± SD. To compare differences and sequential changes in values among three or more groups, statistical analysis was performed using repeated-measures analysis of variance with post hoc Dunnet's tests. Paired Student's t-tests were used for comparison of sequential changes among the values in the same group. $p < 0.05$ was considered significant.

Results

Fig. 1 shows typical currents and summarizes the effects of SEVO-N_2O in the absence and presence of ISO on the peak $I_{Ca,L}$. SEVO-N_2O significantly reduced the peak $I_{Ca,L}$ density by 10.6±6.0% (4.0±1.2 vs. 3.5±1.0 pA/pF; n=13) (**Fig. 1** left). Superfusing cardiac myocytes with 1 mM ISO elicited an approximately 2-fold increase in the $I_{Ca,L}$ amplitude (**Fig. 1** center). In the continued presence of ISO, the reduction in the peak $I_{Ca,L}$ in the presence of SEVO-N_2O was not significant (3.4±6.7%; 13.5±3.5 vs. 13.1±3.5 pA/pF; n=15). Moreover, in the presence of 10 mM FSK, SEVO-N_2O did not reduce the peak $I_{Ca,L}$ significantly (**Fig. 1** right, − 0.8±1.8%; 14.09±0.75 vs. 14.16±0.72 pA/pF; p=0.946, n=12).

Similarly, HALO-N_2O significantly reduced the peak $I_{Ca,L}$ density by 14.7±2.8% (4.5±1.0 vs. 3.8±0.9 pA/pF; n=10). In the presence of ISO, HALO-N_2O significantly reduced the peak $I_{Ca,L}$ by 11.0±3.3% (14.4±3.0 vs. 12.8±3.0 pA/pF; n=11). In the presence of FSK, HALO-N_2O also significantly reduced the peak $I_{Ca,L}$ by 8.1±2.3% (n=7) (**Fig. 2**).

Under basal condition, SEVO and HALO with N_2O induced significant reductions in $I_{Ca,L}$ (**Fig. 3A, B**). In the presence of ISO or FSK, however, the peak $I_{Ca,L}$ densities were not significantly affected by SEVO-N_2O. Those evoked reductions in the peak $I_{Ca,L}$ were clearly smaller than in its absence. **Fig. 3B** indicates that in the presence of ISO or FSK, the reductions in the peak $I_{Ca,L}$ elicited by HALO-N_2O were

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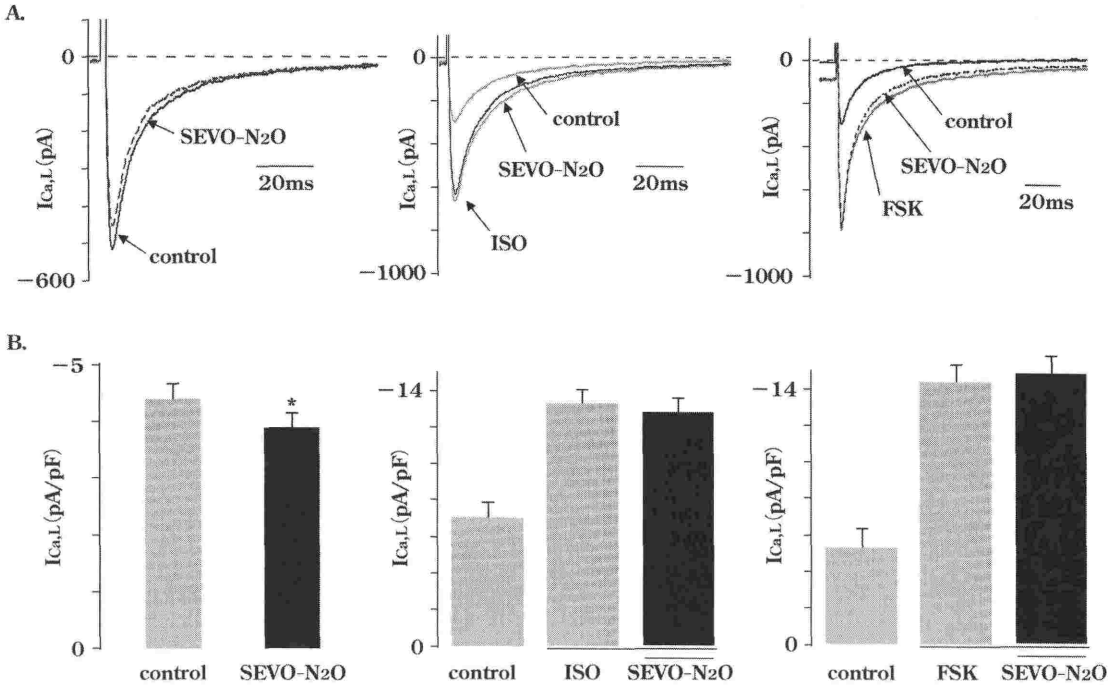

Figure 1 Effects of SEVO-N_2O on peak $I_{Ca,L}$ in the absence (left) and presence (right) of ISO

A: Representative traces of $I_{Ca,L}$ elicited by depolarization to 0 mV from a holding potential of −80 mV, before and after exposure to superfusate saturated with SEVO-N_2O. B: Group data for peak $I_{Ca,L}$ recorded before (control) and after administration of SEVO-N_2O. Bars depict the means±SEM; * $p<0.01$ vs. control.

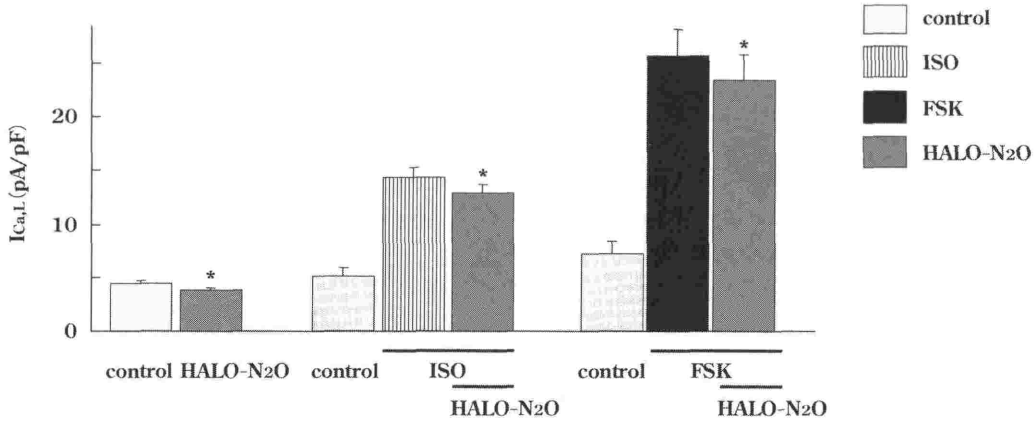

Figure 2 Effects of HALO-N_2O on peak $I_{Ca,L}$ in the absence and presence of ISO or FSK

$I_{Ca,L}$ was significantly reduced by administration of HALO-N_2O in both the absence and presence of 1 mM ISO or 10 mM FSK. Bars depict the means±SEM; * $p<0.01$ vs. $I_{Ca,L}$ recorded before the exposure to HALO-N_2O.

significantly larger than those elicited by SEVO-N_2O. As with SEVO, the reductions in the peak $I_{Ca,L}$ elicited by HALO-N_2O were smaller in the presence of ISO or FSK.

Discussion

In the present study, we compared the effects of SEVO and HALO with N_2O on the peak $I_{Ca,L}$ under basal conditions with the effects of the anesthetics in the presence of ISO or FSK. The capacity of experi-

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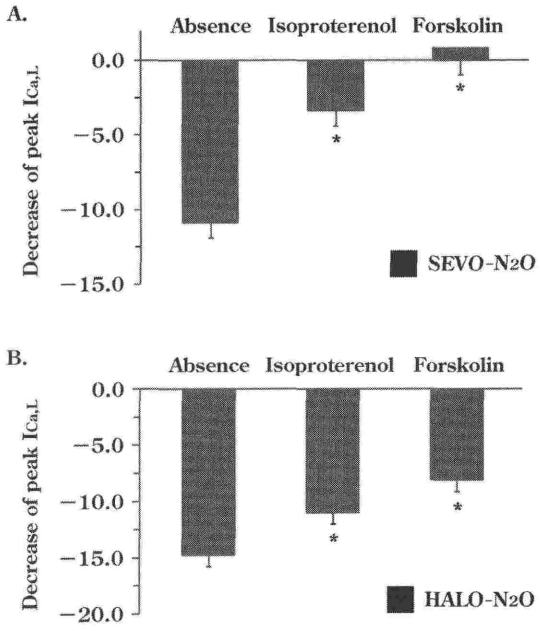

Figure 3 Effects of SEVO and HALO with N_2O on peak $I_{Ca,L}$ in the absence and presence of ISO or FSK

A: Percentage reduction in peak $I_{Ca,L}$ elicited by SEVO-N_2O from the amplitude measured prior to their application. The suppressive effect of SEVO-N_2O on peak $I_{Ca,L}$ significantly diminished in the presence of either ISO or FSK. Bars depict the means±SEM; * $p<0.05$ vs. the absence of ISO or FSK. **B**: Percentage reduction in peak $I_{Ca,L}$ elicited by HALO-N_2O from the amplitude measured prior to their application. FSK did not modify the suppressive effect of HALO-N_2 on $I_{Ca,L}$. Bars depict the means±SEM; * $p<0.05$ vs. the absence of ISO or FSK. Panel **A** was compiled in part using data shown in **Figs. 1**, while panel **B** was compiled using data from **Fig. 2**.

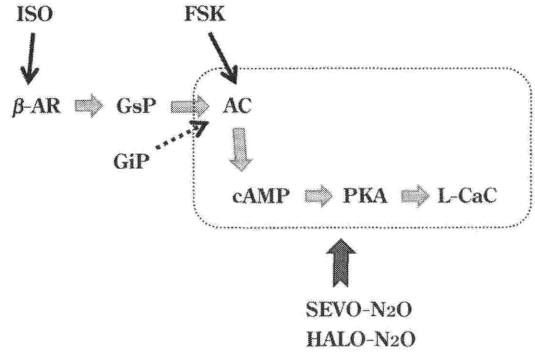

Figure 4 Modulation by HALO-N_2O and SEVO-N_2O on the β-adrenergic signaling pathway

As HALO-N_2O and SEVO-N_2O interact with ISO and FSK, they act downstream of adenylyl cyclase. Narrow arrows indicate the stimulation (↘) and the inhibition (⇢). Wide arrow (⇧) depicts the modulation. β-AR, GsP, GiP, AC, cAMP, PKA, and L-CaC means β-adrenoceptor, Gs protein, Gi protein, adenylyl cyclase, cyclic AMP, and L-type calcium channel.

mental gases to suppress $I_{Ca,L}$ was diminished significantly by the application of ISO or FSK. These results suggest that both SEVO and HALO with N_2O augment β-adrenergic signaling. SEVO and HALO with N_2O augments β-adrenergic stimulation downstream of adenylyl cyclase activation, as FSK activates adenylyl cyclase directly (**Fig. 4**). SEVO and isoflurane both augment the α- and β-adrenergic-mediated enhancement of rat papillary muscle activity[13], and HALO augments the positive inotropic effect of β-adrenergic receptor stimulation on rat papillary muscle through inhibition of the Gi protein[14,15]. Our electrophysiological findings may reflect the effect of SEVO and HALO in the presence of ISO.

Fassl and colleagues reported that the effect of SEVO on human atrial L-type Ca^{2+} channels in the presence of ISO was not different from the effect under basal conditions[16]. They measured total charge movements, and we measured current densities. HALO reduces peak $I_{Ca,L}$ density more than SEVO or isoflurane[11,12,17], since it exerts a greater negative inotropic effect than SEVO, isoflurane, or desflurane[2,18,19]. These reports suggest that the effects of anesthetics on the peak $I_{Ca,L}$ density correlate well with the degree to which the anesthetics depress cardiac performance. Using guinea pig cardiomyocytes, Camara and colleagues demonstrated a difference in the sensitivity of atrial and ventricular $I_{Ca,L}$ to volatile anesthetics, and described a volatile anesthetic-induced depolarizing shift in the steady-state activation of $I_{Ca,L}$ that was absent in atrial L-type Ca^{2+} channels[20]. Based on their findings, these investigators proposed that VAs modulate L-type Ca^{2+} channels differently in the atria and ventricles. The results in this study did not indicate that sensitization of the myocardium to the arrhythmogenic effects of epinephrine by HALO is greater than those by other

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volatile anesthetics. Further study about the effect of volatile anesthetics on ryanodine receptor and potassium channel etc. is required to resolve that mechanism.

Finally, our results suggest that both SEVO and HALO in the presence of N_2O modulate the catecholamine sensitivity of the β-adrenergic cascade, and that SEVO and HALO with N_2O augments β-adrenergic stimulation downstream of adenylyl cyclase activation.

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